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**Biological Effects of Nitrogen Oxides
A Summary of Animal Exposure Experiments**

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Summary
A ~~Review~~ of
BIOLOGICAL EFFECTS OF NITROGEN OXIDES
--ANIMAL EXPOSURE EXPERIMENT--
[Chisso sankabutsu no seitai eikyō
-- Dobutsu hakuro jikken --]

by

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Annual Meeting

Introduction

Nitrogen oxides which are present in the atmosphere include nitrous oxide (N_2O), nitrogen monoxide (NO), nitrous anhydride (N_2O_3), nitrogen dioxide (NO_2), nitrogen peroxide (N_2O_2) and nitric anhydride (N_2O_5). But apart from NO and NO_2 which are present in large quantities and are known to be harmful to the human body, the other nitrogen oxides are not known, for the time being, to be harmful to the human body at the present levels of their concentrations as atmospheric pollutants. With respect to NO_2 , environmental standards have already been decreed (May, 1973), but in the

~~[Translator's note: The above title, author and his affiliation are repeated at the top of p. 223 of the original.]~~

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although its pollution level exceeds that of NO_2

case of NO , the submission of reports on the environmental standard levels has been delayed due to lack of data concerning the chronic effects of NO_2 in areas of high pollution. Advances in ^{NO} research are consequently being awaited eagerly [1].

In order to clarify the aforementioned chronic effects of NO_2 , we have been conducting animal exposure experiments in an attempt to explore the effects of NO_2 on the respiratory system (trachea, bronch^{us} and lung^s) mainly from the histopathological standpoint [2]. As far as NO is concerned, we have started animal experiments, having achieved a measure of success with the exposure techniques which had presented some problems in the past as we shall describe later on. This report will present a brief summary of the findings we have obtained so far through animal experiments using NO_2 and NO .

I. Effects of NO

In certain areas, atmospheric pollution by NO has by now surpassed that of NO_2 . Although it is already known that NO is harmful, its chronic effects still remain to be clarified. The reasons for this are the technical difficulties involved in animal exposure experiments in which the concentration of NO , normally converted into NO_2 in the atmosphere, must be kept constant, and the difficulty of detecting the effects of NO which, in contrast to NO_2 , does not cause mucosal irritation. Pflesser [3] report^{ed} the detection of such central nervous system symptoms as spasms and paralysis as well as methemoglobinemia in mice exposed to high concentrations of NO and assert^{ed} that its acute toxicity ^{was} ~~is~~ several times as strong as that of NO_2 . In contrast, Gray [4] compar^{ed}es the results of his NO_2 exposure experiment with rats with Pflesser's results and conclude^d that NO_2 ^{was} ~~is~~ several times more harmful than

NO. However, their experiments differ in terms of both the experimental animals and the gas compositions as well as the measurement methods so that their results should not be compared without qualification and, consequently, Gray's conclusion remains doubtful [5].

In carrying out our animal exposure experiment with NO, we proceeded with the experiment after first succeeding in ^{preparing} ~~isolating~~ NO by diluting NO contained in cylinders with air and then filtering the mixture through a layer of soda lime to remove most of the NO₂ formed [6]. It is already known that NO ~~forms strong bonds with (Hb) in vitro~~, and ^{hemoglobin / nitrosylhemoglobin} the (NOHb) thus formed has ^{strongly binds} been detected by the electron spin resonance method (ESR) [7]. As far as the in vivo ~~condition~~ ^{experiment} is concerned, Sancier et al. [7] attempted earlier to detect NOHb in the blood of rats exposed to 10 ppm of NO by means of ESR but they were not successful. As we reported earlier [6], we were able to detect NOHb in the blood of mice and rats exposed to several ppm of NO using the procedure described above. More explicitly, we were able to verify that the amount of NOHb in the blood of exposed animals increased with exposure time, that the amount reached a constant level after about 20 minutes in the case of NO at 10 ppm, and that the amount gradually decreased when the animals were placed in purified air. X Also, the amount of NOHb formed was dependent on the NO concentration according to the results we obtained. The results of this series of experiments suggest that the formation of NOHb involves reactions with NO₂ and NO₂⁻ as well as the ^{binding} ~~bonding~~ of NO and Hb, and that there are ^{interspecies} ~~racial~~ differences affecting the formation of NOHb [8, 9]. It would also seem necessary to evaluate the toxicity of NO in terms of the comprehensive

~~1. We are planning to report on the details during this Conference.~~

effects of NO, NO₂ and NO₂⁻ which are believed to exist together in the body ~~system~~ since one should take into consideration the fact that NO is converted into NO₂ and NO₂⁻ in a ^{aerobic condition} ~~system with oxygen~~ such as the circulatory ^{blood} ~~system~~, and consequently, that its ⁱ ~~bonding~~ reaction with Hb would be more complex than that of CO. In addition, one should also take into account ⁶ ~~bonding~~ with such hemoproteins as ¹⁴⁵⁰ ~~cytochrome~~ in the tissues. ~~We have already confirmed in a preliminary test that anemic conditions which were histopathological in nature occurred in the brain and the heart of acutely exposed mice.~~ This ~~finding indicates that in order to proceed with research into the chronic effects of NO, it is necessary to focus our attention not only on the respiratory system but also on the general effects. We are currently exploring this area.~~

II. Effects of NO₂

At the time we began our exposure experiments ~~in the area of~~ ^{to clarify} the chronic effects of low concentration NO₂ under 1 ppm around 1966, there were not many research reports available so that it was necessary to undertake studies aimed at the re-~~evaluation~~ ^{examination} of the dose-effect relations (Table 1). In addition, we had to conduct the exposure experiment discussed below since it was necessary to obtain criteria and other data regarding actual experimental samples in connection with the problem of how to evaluate what kind of changes in ~~our~~ ^{the} histopathological assessment ~~of the levels~~ ^{as the index} of the chronic effects. Thereafter came various reports on the subject from Blair et al. [10] and others [10 - 13] in ~~quick~~ succession, but so far at least, and as far as small animals (mice and rats) are concerned, the histopathological findings

~~1. We are planning to report on the details during this Conference.~~

we have obtained regarding the chronic effects of NO₂ are ~~more or less~~ in agreement with these reports. on the whole

1.濃度	2.暴露期間	3.動物	4.病理変化	5.研究者(年次)
(1) 1 ppm	6時間/日、毎日、 14-18ヶ月	15.マウス (Sherman) 16.ウツ (C 57, B 6 / 6 C A F ₁ / Sae)	18.気管支慢性炎症と気管支炎	Stokinger (1965)
(2) 0.8 ppm	33ヶ月	16.マウス	19.肺動脈硬化 20.肺動脈炎 21.肺動脈硬化(1)	Freeman (1966)
(3) 0.7-0.8 ppm	30日	17.ウツ (ICR) ♀	22.気管支慢性炎症と気管支炎 23.肺動脈硬化(1)	中島、中島ら (1966)
(4) 2 ppm ± 1 ppm	2日	18.マウス (Sprague-Dawley) ♀	24.気管支慢性炎症と気管支炎 25.肺動脈硬化(1)	Freeman (1968)
(5) 1 ppm 0.5 ppm	10時間 4時間	16.マウス (Sprague-Dawley) ♂	26.肺動脈硬化(1) 27.肺動脈硬化(2) 28.肺動脈硬化(3) 29.肺動脈硬化(4) 30.肺動脈硬化(5) 31.肺動脈硬化(6) 32.肺動脈硬化(7) 33.肺動脈硬化(8) 34.肺動脈硬化(9) 35.肺動脈硬化(10) 36.肺動脈硬化(11) 37.肺動脈硬化(12) 38.肺動脈硬化(13) 39.肺動脈硬化(14) 40.肺動脈硬化(15) 41.肺動脈硬化(16) 42.肺動脈硬化(17) 43.肺動脈硬化(18) 44.肺動脈硬化(19) 45.肺動脈硬化(20) 46.肺動脈硬化(21) 47.肺動脈硬化(22) 48.肺動脈硬化(23) 49.肺動脈硬化(24) 50.肺動脈硬化(25) 51.肺動脈硬化(26) 52.肺動脈硬化(27) 53.肺動脈硬化(28) 54.肺動脈硬化(29) 55.肺動脈硬化(30) 56.肺動脈硬化(31) 57.肺動脈硬化(32) 58.肺動脈硬化(33) 59.肺動脈硬化(34) 60.肺動脈硬化(35) 61.肺動脈硬化(36) 62.肺動脈硬化(37) 63.肺動脈硬化(38) 64.肺動脈硬化(39) 65.肺動脈硬化(40) 66.肺動脈硬化(41) 67.肺動脈硬化(42) 68.肺動脈硬化(43) 69.肺動脈硬化(44) 70.肺動脈硬化(45) 71.肺動脈硬化(46) 72.肺動脈硬化(47) 73.肺動脈硬化(48) 74.肺動脈硬化(49) 75.肺動脈硬化(50) 76.肺動脈硬化(51) 77.肺動脈硬化(52) 78.肺動脈硬化(53) 79.肺動脈硬化(54) 80.肺動脈硬化(55) 81.肺動脈硬化(56) 82.肺動脈硬化(57) 83.肺動脈硬化(58) 84.肺動脈硬化(59) 85.肺動脈硬化(60) 86.肺動脈硬化(61) 87.肺動脈硬化(62) 88.肺動脈硬化(63) 89.肺動脈硬化(64) 90.肺動脈硬化(65) 91.肺動脈硬化(66) 92.肺動脈硬化(67) 93.肺動脈硬化(68) 94.肺動脈硬化(69) 95.肺動脈硬化(70) 96.肺動脈硬化(71) 97.肺動脈硬化(72) 98.肺動脈硬化(73) 99.肺動脈硬化(74) 100.肺動脈硬化(75) 101.肺動脈硬化(76) 102.肺動脈硬化(77) 103.肺動脈硬化(78) 104.肺動脈硬化(79) 105.肺動脈硬化(80) 106.肺動脈硬化(81) 107.肺動脈硬化(82) 108.肺動脈硬化(83) 109.肺動脈硬化(84) 110.肺動脈硬化(85) 111.肺動脈硬化(86) 112.肺動脈硬化(87) 113.肺動脈硬化(88) 114.肺動脈硬化(89) 115.肺動脈硬化(90) 116.肺動脈硬化(91) 117.肺動脈硬化(92) 118.肺動脈硬化(93) 119.肺動脈硬化(94) 120.肺動脈硬化(95) 121.肺動脈硬化(96) 122.肺動脈硬化(97) 123.肺動脈硬化(98) 124.肺動脈硬化(99) 125.肺動脈硬化(100)	Thomas (1967)
(6) 0.5 ppm	13-12ヶ月 6、18、24時間/日 連続 (7日/週) 6時間 × 3ヶ月 24時間 × 3ヶ月	17.ウツ (Swiss Albino) ♀	23.肺動脈硬化(1) 24.肺動脈硬化(2) 25.肺動脈硬化(3) 26.肺動脈硬化(4) 27.肺動脈硬化(5) 28.肺動脈硬化(6) 29.肺動脈硬化(7) 30.肺動脈硬化(8) 31.肺動脈硬化(9) 32.肺動脈硬化(10) 33.肺動脈硬化(11) 34.肺動脈硬化(12) 35.肺動脈硬化(13) 36.肺動脈硬化(14) 37.肺動脈硬化(15) 38.肺動脈硬化(16) 39.肺動脈硬化(17) 40.肺動脈硬化(18) 41.肺動脈硬化(19) 42.肺動脈硬化(20) 43.肺動脈硬化(21) 44.肺動脈硬化(22) 45.肺動脈硬化(23) 46.肺動脈硬化(24) 47.肺動脈硬化(25) 48.肺動脈硬化(26) 49.肺動脈硬化(27) 50.肺動脈硬化(28) 51.肺動脈硬化(29) 52.肺動脈硬化(30) 53.肺動脈硬化(31) 54.肺動脈硬化(32) 55.肺動脈硬化(33) 56.肺動脈硬化(34) 57.肺動脈硬化(35) 58.肺動脈硬化(36) 59.肺動脈硬化(37) 60.肺動脈硬化(38) 61.肺動脈硬化(39) 62.肺動脈硬化(40) 63.肺動脈硬化(41) 64.肺動脈硬化(42) 65.肺動脈硬化(43) 66.肺動脈硬化(44) 67.肺動脈硬化(45) 68.肺動脈硬化(46) 69.肺動脈硬化(47) 70.肺動脈硬化(48) 71.肺動脈硬化(49) 72.肺動脈硬化(50) 73.肺動脈硬化(51) 74.肺動脈硬化(52) 75.肺動脈硬化(53) 76.肺動脈硬化(54) 77.肺動脈硬化(55) 78.肺動脈硬化(56) 79.肺動脈硬化(57) 80.肺動脈硬化(58) 81.肺動脈硬化(59) 82.肺動脈硬化(60) 83.肺動脈硬化(61) 84.肺動脈硬化(62) 85.肺動脈硬化(63) 86.肺動脈硬化(64) 87.肺動脈硬化(65) 88.肺動脈硬化(66) 89.肺動脈硬化(67) 90.肺動脈硬化(68) 91.肺動脈硬化(69) 92.肺動脈硬化(70) 93.肺動脈硬化(71) 94.肺動脈硬化(72) 95.肺動脈硬化(73) 96.肺動脈硬化(74) 97.肺動脈硬化(75) 98.肺動脈硬化(76) 99.肺動脈硬化(77) 100.肺動脈硬化(78) 101.肺動脈硬化(79) 102.肺動脈硬化(80) 103.肺動脈硬化(81) 104.肺動脈硬化(82) 105.肺動脈硬化(83) 106.肺動脈硬化(84) 107.肺動脈硬化(85) 108.肺動脈硬化(86) 109.肺動脈硬化(87) 110.肺動脈硬化(88) 111.肺動脈硬化(89) 112.肺動脈硬化(90) 113.肺動脈硬化(91) 114.肺動脈硬化(92) 115.肺動脈硬化(93) 116.肺動脈硬化(94) 117.肺動脈硬化(95) 118.肺動脈硬化(96) 119.肺動脈硬化(97) 120.肺動脈硬化(98) 121.肺動脈硬化(99) 122.肺動脈硬化(100)	Ehrlich (1969)
(7) 0.5-0.8 ppm	1ヶ月連続	17.ウツ (ICR) ♂ (dd) ♀	29.気管支慢性炎症と気管支炎 30.肺動脈硬化(1)	中島、中島ら (1970)
(8) 0.5-1.0 ppm	13日連続暴露後 インフルエンザ (PR8) 吸入感染	17.ウツ (ICR) ♂	31.気管支慢性炎症と気管支炎 32.肺動脈硬化(1)	中島、中島ら (1971)
(9) 1.0-1.5 ppm	1ヶ月連続後 清浄空気 1-3ヶ月	17.ウツ (ICR) ♂	33.気管支慢性炎症と気管支炎 34.肺動脈硬化(1)	中島、中島ら (1972)
(10) 0.3-0.5 ppm	7ヶ月連続暴露後 インフルエンザ (PR8) 吸入感染	17.ウツ (ICR) ♂	35.気管支慢性炎症と気管支炎 36.肺動脈硬化(1)	中島、中島ら (1972)

Table 1. Reports concerning histopathological effects of low concentration NO₂.

- Key: 1. Concentration
2. Exposure time duration
3. Animal
4. Pathological change
5. Researchers (chronological order)
6. 6 hrs/day, daily, 14 - 18 months
7. 33 months
8. 30 days
9. 2 years
10. 2 hrs, 4 hrs
11. 3 - 12 months
6, 18, 24 hrs/day
continuous (7 days/week)
6 hrs x 3 months
24 hrs x 3 months

(continued on next page)

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12. 1 month continuously
13. Influenza (PR 8) infection by inhalation after 39 days of continuous exposure
14. 1 month continuously
1 - 3 months of pure air
15. Influenza (PR 8) infection by inhalation after 6 months of continuous exposure
16. Rats
17. Mice *Obstructive*
18. ~~Obliterating~~ fibrous bronchiolitis (-)
19. Pneumonitis (-)
Pulmonary emphysema (-)
Enlarged alveolar cavity (+)
20. Catarrhal bronchitis (+)
Enlarged alveolar cavity (+)
21. Diminution or loss of cilia (+)
~~Red shaped, crystalline plastids in cytoplasm~~ (+ by electron microscope)
Cold inclusion body of ~~disorientation~~
22. ~~Clasmotosis~~ of mast cells in lung, and loss ~~and configuration~~
~~disarray of~~ dytoplasmic granules (with recovery 24 hours after termination of exposure)
23. Enlarged alveolar cavity (+)
Bronchiolitis (+)
Rupture Local ~~Partial~~ pulmonary emphysema (+)
Lymphocyte infiltration of interstices around bronchia (+)
Bronchiole obstruction (+)
Enlarged alveolar cavity (+)
24. Thickened and shortened bronchial cilia, edematous swelling of alveoli *proliferation*
25. Adenomatous ~~heteroforms~~ of bronchial epithelial cells *proliferation*
26. *Picture* ~~Image~~ of chronic bronchitis in trachea and bronchia
27. Proliferation of lymphatic tissues
Lymphomatous ~~condition~~
28. ~~et al.~~ *change*
29. Nakajima, Chen, et al.
30. Nakajima, Hattori, et al.
31. Itō, Nakajima, et al.
32. Chen, Nakajima, et al.

Experimental Procedure

Gas exposure method [15]: We used the exposure chamber (made of stainless steel) shown in Photo 1. For the NO₂ gas supply, we used NO₂ gas generated by heating liquid N₂O₄. The gas was diluted with purified air before ^bbeing introduced via an automatic concentration regulation device

[16] (Fig. 1) into the chamber wherein the animals were exposed continuously, 24 hours a day. The concentration was measured with a continuous automatic NO_2 monitor (Saltzman ^{VS} type, batch method) with a conversion coefficient of 0.72. For the experimental animals, we used JCL-ICR mice (some of them ddO) which were 3 - 4 weeks old. The control group animals were kept in another chamber of the same model equipped with an air purification device and were left to take in the feed and water freely.

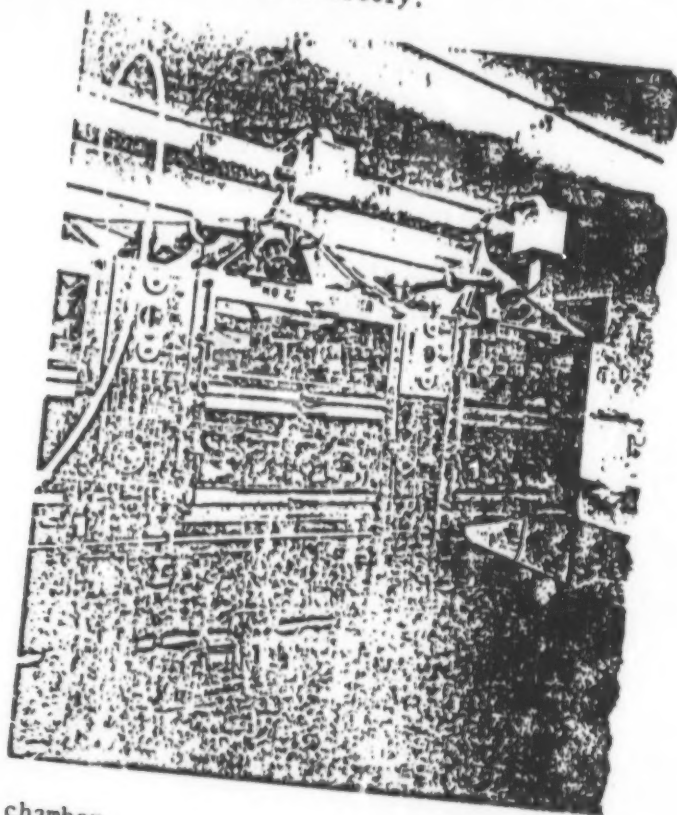


Photo 1. Exposure chamber

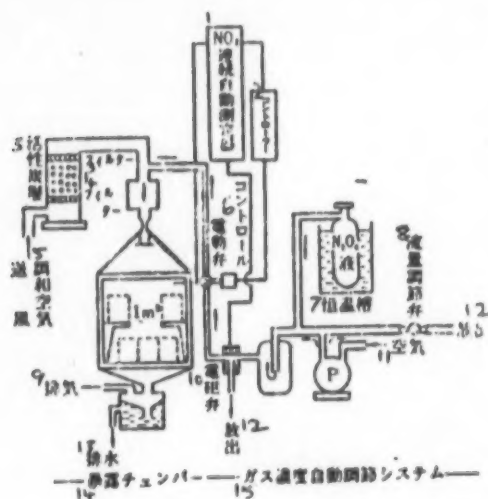


Fig. 1. Schematic diagram of low concentration NO_2 exposure system.

- Key:
1. Continuous automatic NO_2 monitor
 2. Controller
 3. Activated charcoal filter
 4. Filter
 5. Conditioned air ~~blower~~
 6. Electrical control valve
 7. Thermostatic tank
 8. Flow regulation valve
 9. Exhaust
 10. Electromagnetic valve
 11. Air
 12. Discharge
 13. Water discharge
 14. Exposure chamber
 15. Automatic gas concentration regulation system

Detection method: For ~~our~~ optical microscope examinations [17, 18], we killed the mice by the cervical vertebra dislocation ~~method~~ and ^{removed} ~~extracted~~ their lungs after ~~first~~ injecting a 10% formalin solution from the trachea. The examinations were carried out using H. E. stain, elastic fiber stain, PAS stain, Azan's and Mallory's stains, Van Gieson's stain and ^{silver-stain} ~~argentaion~~. In the preparation of the electron microscope samples, tissues from the

bronchia and the lungs were fixed by the method reported previously [19], after which they were ^{embedded} ~~packed~~ in epon ~~(X)~~ [Japanese: ~~epon~~] and stained with uranyl acetic ^{ate} ~~acid~~ and lead ^{acetate} ~~acetate~~.

Influenza virus ^{inhalation} infection method [18]: The mice were placed in individual cages and then ^{infected by} ~~sprayed~~ ^{virus suspension} with a nebulizer inside a desiccator. For the virus, we used a strain of PR 8 [influenza virus A/PR/8/34 (H1N1)]. Incidentally, the ^{contamination} ~~invaccination~~ of HVJ (para influenza virus type I) was ruled out as a result of ^{hem} ~~red cell~~ agglutination test.

Results

1. Histopathological Changes in Trachea, Bronchia and Lungs

In the trachea of mice (3-week-old, ♀) exposed to 0.7 - 0.8 ppm for 1 month [17], vacuol^uer degeneration, hypersecretion, desquamation and ^{decomposition} ~~clasma~~ ~~tosis~~ of the mucous ^{membrane} ~~epithelia~~ were observed locally on the mucous layers (Photo 2). Also, light congestion was observed in the submucous ^{sa} ~~coats~~. In the mucous layers, the degeneration ~~image~~ was found to become more marked with the proliferation of the mucous ^{membrane} ~~epithelia~~, while the intercellular spaces were found to be significantly enlarged and pyknosis was also prominent. We were also able to observe clearly the desquamation of the degenerated cells which covered the surface of the mucous membranes together with mucous secretions. In the submucous tissues, dilation of the small blood vessels and light edema were also observed. In the lung tissues, there was hypersecretion of ^{mucus} ~~mucilage~~ as well as inflammatory bronchitis. In the bronchioli, proliferation of the mucous ^{membrane} ~~epithelia~~, hypersecretion, degeneration and desquamation were observed clearly. Due to this condition, the bronchiolar cavities were found to be filled with mucous secretions and desquamative

epithelia. The small blood vessels showed a dilation of the vascular cavities and ^{filling} ~~repletion~~ of red ^{blood} cells. In the alveolar septa, very slight infiltration by monocytes was detected together with a slight dilation of the capillary vessels. The sizes of the alveolar cavities were found to be uneven with some showing enlargement (Photo 3). These changes were not observed in the control group.



Photo 2. Degeneration and desquamation of bronchial mucous membrane.

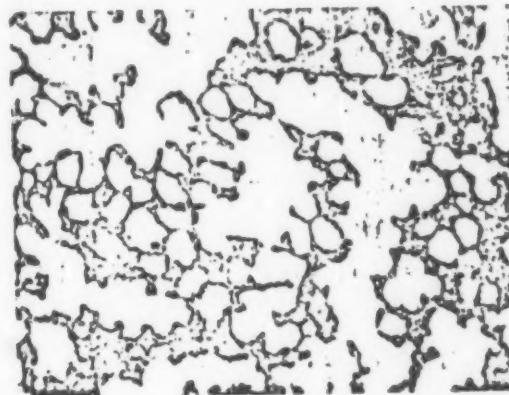


Photo 3. Enlargement of alveolar cavities

The morphological changes occurring in the bronchial-alveolar system of mice (ddO, 4-week-old, ♀) exposed to 0.5 - 0.8 ppm for 1 month were studied in detail with an electron microscope [19]. In the bronchia, edematous swelling of the membrane on the surface of the cilia on the ciliated epithelial cells (which is to say the widening of the ~~anhistous~~ regions between the fiber bundles and the surface) as well as the shortening of the cilia were observed (Photo 4). There was no change in the ~~ciliate~~ ^{plet} roots. Edematous ^{change} ~~condition~~ was also partially observed in the ~~clara~~ ^{clara} [clara or curare (?)] ~~cytoplasm~~. With respect to cells, edematization was also observed in the ~~epithelial~~ ^{cells} cytoplasm and intercellular substance of the small [?] ~~alveoli~~

alveolar epithelial cells

(Photo 5). ^{These} ~~This~~ changes exhibited a tendency of being more pronounced in the alveolar epithelial cells closer to the terminal bronchia. Indications similar to those described above were also found in mice (4-week-old, ♂) exposed to 0.5 - 1.0 ppm for 1 month [18]. Histological changes in mice exposed continuously to 0.3 - 0.5 ppm for 6 months were also examined after 3 months and at the end of the 6-month period [20]. It was found that the inflammatory condition due to the mucous membrane irritation effect of NO₂ was light, with principal changes consisting of proliferative ^{changes} ~~condition~~ of the bronchial epithelial cells together with hyperplasia of lymphatic nodules around them and around the blood vessels as we shall discuss below.

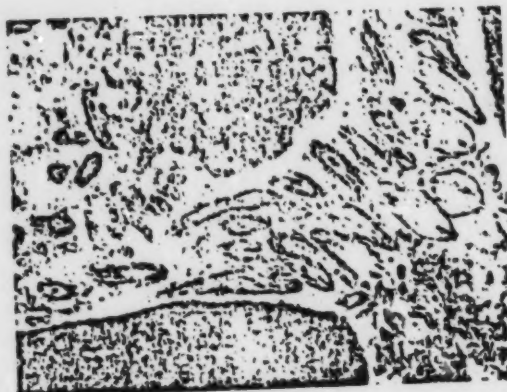


Photo 4. Edematous swelling of ~~cilia~~ ^{cilia} (x 10000)

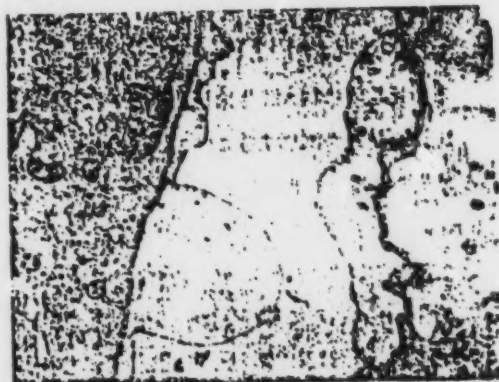


Photo 5. Edematous swelling of alveolus (x 10000)
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2. Proliferative Changes of Bronchial Epithelial Cells

It is worth noting that a proliferative change of the ^{bronchial} ~~tracheal~~ epithelial cells, which is to say the condition of so-called hyperplastic foci (Photo 6), and hyperplasia of the lymphatic nodules around them (Photo 7) were observed in the lungs of mice exposed to 0.5 - 0.8 ppm and 0.5 - 1.0 ppm for about 1 month, and to 0.3 - 0.5 ppm for 6 months respectively, as we mentioned above.

The proliferative change of the epithelial cells was more pronounced in the peripheral bronchia than in the ^{larger} ~~main~~ bronchi. Most of the epithelial cells in the peripheral bronchia were ^{tall} ~~long~~ and ^{columnar} ~~cylindrical~~ and consisted of cytoplasm which stained well with eosin and nucleus which were mostly ^{elliptical} ~~oval~~ shaped. Most cells ^{formed} ~~showed~~ regular ^{mono layer} ~~configuration of rows~~, but in some places, we observed that the epithelial cells were stratified in such a way that they protruded like ^{papilla} ~~minimization~~ ^{on} from the bronchial lining and, in some cases, swelled up ~~as a result of~~ ^{by} papillematous proliferation together with the submucous tissues (3/12, Photo 8).



Photo 6. ~~Stratified~~ Hyperplasia of terminal bronchial epithelia (hyperplastic foci) and cuboid metaplasia of bronchiolar epithelia.

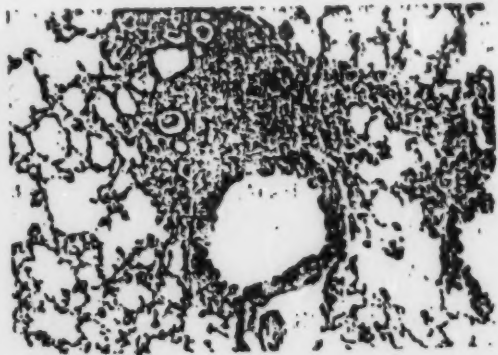


Photo 7. Proliferation of lymphatic follicles around peripheral bronchia



Photo 8. ^{any} Papillematous proliferation of bronchial epithelial cells

The electron microscope^{ic} examination of the proliferative changes described above showed that most of the peripheral bronchial cells proliferating toward the alveoli were ~~alveolar~~^{Clara} cells [19].

In terms of the chronic effects of NO_2 , it is well known that Ehrlich et al. [21] have reported higher mortality rate due to infection by Klebsiella pneumoniae and influenza virus, as well as weakened resistance to ~~the~~^{respiratory} ~~chert~~ infection due to such factors as diminished rejection ~~capacity~~^{ability} against living bacteria. In addition, Kusano, in his general ~~remarks~~^{review} on the pathology of influenza [22], described in detail the occurrence of adenomatous proliferation of bronchial epithelial cells in the alveolar cavity of mice infected with influenza virus. We ourselves also observed the adenomatous proliferation of the peripheral bronchial epithelial cells in a group of exposed mice which had been infected with influenza virus for the purpose of studying the histopathological aspects of the diminished resistance to ~~tracheal~~^{respiratory} infection due to exposure. ~~To wit,~~^{namely} only the papilloma^{ary} ~~tous~~ proliferation of the peripheral bronchial epithelial cells occurred in mice exposed only to 0.5 - 1.0 ppm of NO_2 for 1 month, and adenomatous proliferation of the peripheral bronchia occurred in only a small number (1/12) of mice subjected solely to influenza infection, whereas the incidence of adenomatous proliferation was higher than either of the above two (7/12) in the case of mice which were infected with influenza after they had been exposed to NO_2 (Photo 9). As far as our observation is concerned, however, there is no positive evidence for the time being that this adenomatous proliferation would become malignant since we could not detect a ~~clear image~~^{appearance} of the destruction of the elastic fibers in the proliferation ~~images~~^{pictures} [18].



Photo 9. Adenomatous proliferation of bronchial epithelial cells.

The most notable change observed in the group infected with influenza virus after 3 months of exposure to 0.3 - 0.5 ppm of NO_2 in comparison with the non-exposed infected group was that the former exhibited a more advanced inflammatory condition and a proliferation of the lymphatic tissues [20]. ^{namely} ~~To elaborate~~, advanced edemas were observed in the connective tissues around the bronchia and on the bronchial arteries where infiltration of the cells was pronounced, and infiltration of cells was also detected continuously toward the alveolar septa. The bronchial epithelial cells were found to consist of cuboid cells while the cytoplasm was somewhat basophilic and their nuclei were ^{round} ~~circular~~. Also, the superficial epithelial cells were squamatized although no proliferative change of the epithelia could be detected. Around the bronchial arteries and around the bronchia themselves, proliferation of the lymphatic follicles was observed together with nodular proliferation and lymphadenomatous proliferation [21]. In a group infected with influenza after 6 months of exposure, the ^{picture} ~~image~~ of inflammation was more or less of the same degree as that of the group infected after 3 months of exposure, but the proliferation of the lymphatic tissues around the bronchia and the

blood vessels were more pronounced in the former group. ^{namely} ~~To elaborate~~, there was a hyperplasia of lymphatic follicles around the bronchia and the blood vessels, and nodular proliferation and lymphadenomatous proliferation were found to be more pronounced in the latter group. The ^{incidence} ~~extents~~ of the proliferation of the peripheral bronchial epithelial cells ~~and the incidences of~~ lymphatic follicles, and the extents of interstitial pneumonia for the different groups are shown in Tables 2 and 3 [21].

1グループ	2匹数	3末梢気管支上皮細胞の増殖				4リンパ球の増殖				5間質性肺炎
		A	P	S	none	L	N	LF	none	
6月 照	7	0	0	1	6	0	1	5	1	—
7月 露	17	1	5	7	4	3	2	11	1	—
8月 暴露感染	3					0	2	1	0	軽度
9月 露感染	2					1	5	6	0	高度

Table 2. NO₂ 0.3 - 0.5 ppm (3 months)

1グループ	2匹数	3末梢気管支上皮細胞の増殖				4リンパ球の増殖				5間質性肺炎
		A	P	S	none	L	N	LF	none	
6月 照	5	0	0	2	3	0	0	4	1	—
7月 露	14	1	6	7	0	4	2	8	0	—
8月 暴露感染	5					0	3	2	0	軽度
9月 露感染	10					2	5	3	0	高度

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A: 腺腫様増殖 P: 乳頭状増殖
S: 絨毛の増殖 L: リンパ腺腫様増殖
N: 結節状増殖 LF: リンパ球

Table 3. NO₂ 0.3 - 0.5 ppm (6 months)

- Key: 1. Group
2. Number of animals
3. Proliferation of peripheral bronchial epithelial cells
4. Proliferation of lymphatic follicles
5. Interstitial pneumonia
6. Control
7. Exposed
8. Non-exposed, infected
9. Exposed, infected
10. Light
11. Advanced
12. A: Adenomatous proliferation P: Papill^{ary}omatous proliferation
S: Stratified proliferation L: Lymphadenomatous proliferation
N: Nodular proliferation LF: Lymphatic follicles

3. Occurrence of Chronic Bronchitis ~~image~~

For the purpose of observing how the respiratory ^{regions caused by} ~~system afflicted due~~ to NO₂ exposure ^{would recover after} ~~reintegrates itself once~~ the exposure is halted, we conducted a histopathological examination of mice kept in an ambience of purified air after exposure to NO₂. We observed a remarkable fluctuation in the pathological conditions including, notably, the occurrence of ^{pictures} ~~traces~~ of chronic tracheitis and chronic bronchitis [23].

The most notable finding regarding the trachea of mice which were kept in purified air for 1 month after exposures of 1 month to 1.0 - 1.5 ppm and 6 months to 0.3 - 0.5 ppm was that there was pronounced infiltration by monocytes and lymphocytes. This infiltration by monocytes and lymphocytes was also marked in the submucous tissues (Photo 10). Apart from the monocytes and the lymphocytes, a small number of plasma cells and histiocytes were also observed. In some cases, the state of infiltration exhibited lymphatic follicle-like structures. In the alveolar septa, slight dilations of the capillary vessels were seen but inflammatory cells could not be detected. Such infiltration by lymphocytes was found to have either abated or disappeared altogether after the mice had been kept for 3 months in purified air. Around the bronchioles in the lungs, we also observed infiltration by lymphocytes which either surrounded the bronchioles completely or were localized to parts of areas surrounding them. Lymphocyte infiltration thus surrounded the respiratory tracts in many cases, but in some cases, they were observed as ^{islet in form} ~~clusters~~ around the bronchioles or around the small blood vessels.



Photo 10. Lymphocyte infiltration around bronchium^{us}

Such ^{pictures} ~~indications~~ as those described above, in fact, resemble ^{that} ~~tissue~~ ^{e. disease} ~~symptoms~~ closely, and are rather different from the ~~images~~ ^{pictures} of auto-immunity ~~images~~ of degeneration, desquamation and ^{decomposition} ~~clasmatosis~~ of the mucous epithelia which are seen either during or immediately after exposure to NO₂. This occurrence of ^{these pictures} ~~symptoms~~ principally involving lymphocytic response may conceivably be explained as follows. While a part of the ^{generated} ~~des~~ ^{membrane in} ~~epithelia~~ from the trachea and the bronchi ~~quamatized~~ and decomposed mucous ^{epithelia} ~~from~~ the trachea and the bronchi is excreted as sputum, another part acts on the trachea and the bronchi to cause an antigen-antibody reaction.

4. Fluctuation in Level of Reductive^{ed} Glutathione in Lung Tissue, and Compound Effects with CO

The fluctuation in the level of reductive^{ad} glutathione (GSH), which is believed to take part in the maintenance of the cell wall structure, may be considered to reflect a state of disturbance in the cell membrane. The GSH level in the mouse lung tissue first declined after several days of exposure (0.8 ± 0.04 ppm, 5 days), but thereafter, it recovered to remain at a level of no significant difference from that of the control group ($0.7 -$

0.8 ppm, 1 month). However, after a period at which the body weight of the exposed group became lighter than that of the control group, the GSH level of the former was observed to be lower than that of the control group [2, 15, 17].

Exposed group (n = 9) 634 ± 139 $\mu\text{g/lung wet weight}$

Control group (n = 11) 875 ± 130 $\mu\text{g/lung wet weight}$

(p < 0.01)

The above phenomenon may conceivably be explained as follows. The lung GSH level declines once after a short period of exposure but soon regains the level prior to exposure due to GSH transfer to the lungs from organs such as the liver caused by the ~~body's~~ biological responses ~~and other factors~~. Thereafter a period elapses during which the GSH level is ^{compensatorily} maintained at normal due to the ~~body's~~ increased ^{air} biosynthetic capacity, but eventually, when a phase arrives at which ^{decrease of} a body weight difference in comparison the control group occurs with the progression of the chronic effects, the GSH level declines.

It is believed that the absorption and elimination of CO may be determined by the partial pressures of CO and O₂ in expiratory air and are affected by such factors as the gas exchanging capacity, the gas ventilation rate and the ~~blood~~ ^{volume} flow of the lungs ^{blood circulation}. Murphy et al. [26] report ^{ed} that the acute effects of irradiated exhaust gases ^{heightens} include an increase ^{of} in the COHb level, but we could not detect any ^{enhancement of} COHb increase in the mice (4-week-old, $\frac{1}{2}$ $\frac{0}{0}$, $\frac{0}{4}$) we exposed to a mixture of low-concentration CO and NO₂ (CO 50 ppm, NO₂ 0.5 - 0.8 ppm). The result of our experiment, in fact, agreed with those of the subacute combined exposure experiments of Lutmer et al. [24] with respect to CO combinations with NO ^{or} and NO₂ [32].

Summary

I. Although information regarding the chronic effects of NO is still rather scant at present, it is nevertheless evident that NO is harmful, and furthermore, pollution by NO has by now surpassed that of NO₂. In view of this situation, it is essential that the nature of NO pollution be explained as soon as possible. The author pointed out earlier that NO, unlike NO₂, must be considered in terms of its general effects as well. Since NO may be converted to NO₂ in the respiratory tract, there could also be effects on the respiratory ^{organs} ~~apparatus~~. In this connection, one must await the clarification of the conversion rate of NO to NO₂ in the respiratory tract before knowing how much of the NO can be evaluated as NO₂.

II. Our histopathological findings regarding the chronic effects of NO₂ were: 1) Disorders due to its irritative action on the ^{bronchial} ~~tracheal~~ mucous membrane and the alveoli, and the occurrence of the chronic bronchitis ^{picture} ~~image~~ ~~which is apparently caused by the said disorders~~; 2) ~~notable~~ ^{proliferative} change in the epithelial cells of the terminal bronchia; and 3) enlargement of the alveolar cavity. Since NO₂ ^{is a deep irritant} ~~has a pervasive property~~ in the lungs, it is believed that the gas has stronger effects in the deep lung regions than does SO₂. The histological indications of the effects 1) and 3) above are that they can cause ^{obstructive} ~~obliterative~~ respiratory ~~system~~ disorders as can the sulfur oxides. At the same time, these effects are considered to be the cause behind the fact that the number of ^{the} ~~officially recognized~~ patients suffering from air pollution ~~afflictions~~ continues to climb in regions where the SO₂ ~~pollutant~~ concentration level is known to be declining.

Proliferative changes in the bronchial epithelial cells due to exposure to relatively low concentrations have been reported in guinea pigs by Storkinger et al. [10], in mice by Loosli et al. [25], in rats by Freeman et al. [35], in rabbits by Hydon et al. [36], and in mice infected with influenza virus after exposure and monkeys by Ehrlich et al. [14, 21, 27]. With respect to alveolar septum cells, Freeman et al. [37] and Sherwin et al. [38] observed that hyperplasia of Type II cells occurs in order to compensate for the Type I cells damaged by NO_2 . As far as the possible link to lung cancer is concerned, there is no positive evidence that such proliferative changes become malignant. ^{Although the} ~~but~~ results of animal experiments should not be applied without qualification to human beings, ~~furthermore~~, the question of whether or not there is a carcinogenic property ought to be settled experimentally using mice exposed to NO_2 . At any rate, these effects of NO_2 should be considered as significant in view of the fact that carcinogenic factors and infection factors are ~~already known to be~~ present not only in the atmosphere but in the living environment in general.

In addition to these histopathological effects, other biological effects of NO_2 have been observed in experimental animals, including biochemical effects on the ^{lung} ~~surface~~ ^{tant} ~~active substances of the lungs~~ [29], weakening of the biological defense mechanisms against ^{respiratory} ~~bronchial~~ infection [30], and diminution in the ~~body's~~ antibody producing capacity [31, 33, 34]. So far, the lowest experimental concentration of NO_2 at which chronic effects have been recognized in experimental animals has been around the 0.5 ppm level. It is hoped that studies in greater detail will be made in the future in to the effects of even lower concentrations and into combined effects with other pollutants with the development of more sensitive effect indices.

~~It should be noted~~ ^{studies} The ^{done} results reported here were ^{project} obtained in a ^{joint} research with ^{Mits} Shigeko Kusumoto and Hajime Oda (~~Environmental Pollution Hy-~~
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~~(Department of Nutritional Pathology, Faculty of Home Economics, Kyoto~~
Women's College), ^{Shoji} ~~Masaji~~ Hattori and Ryūhei Tateishi (~~Osaka Prefectural~~
Center of Adult Diseases), ^{Osaka} ~~Makoto~~ Yoshida, Ken Motomiya and Kōzō Itō (^{Kyo} Depart-
ment of Public Health, Chiba University Medical School), and Genshirō Ide
and Yūji Otsu (Department of Pathology, Chiba University Medical School).

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